PB82-119488

# Recommended Guideline for Teratogenicity Studies in the Rat, Mouse, Hamster, or Rabbit

(U.S.) Interagency Regulatory Liaison Group
Washington, DC

Jan 81

U.S. Department of Commerce
National Technical Information Service
NTIS

PB82-119488

January 1981

# Interagency Regulatory Liaison Group

**TESTING STANDARDS AND GUIDELINES WORK GROUP**

REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA 22161

**Recommended Guideline For**

# TERATOGENICITY STUDIES IN THE RAT, MOUSE, HAMSTER, OR RABBIT

**U.S. Consumer Product Safety Commission**
**U.S. Environmental Protection Agency**
**Food and Drug Administration (U.S. Dept. of HHS)**
**Food Safety and Quality Service (U.S. Dept. of Agriculture)**
**Occupational Safety and Health Administration (U.S. Dept. of Labor)**

BIBLIOGRAPHIC INFORMATION

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PERFORMER: Interagency Regulatory Liaison Group, Washington, DC.

This guideline is for use with substances given orally to the rat, mouse, hamster, or rabbit. The purpose of this test is to yield data to help determine whether a test substance is potentially embryolethal and/or teratogenic. Treatment must be started early enough and continued long enough to include the period of organogenesis for the particular species used.

KEYWORDS: *Toxicology, *Teratogenesis.

Available from the National Technical Information Service, Springfield, Va. 22161

PRICE CODE: PC A02/MF A01

## FOREWORD

Four regulatory agencies, the Consumer Product Safety Commission **(CPSC)**, the Environmental Protection Agency **(EPA)**, the Food and Drug Administration **(FDA)**, and the Occupational Safety and Health Administration **(OSHA)**, agreed to work together to reform the regulatory process and to improve protection of workers, public health, and the environment **(42 FR 54856, 11 October 1977)**. They formed the Interagency Regulatory Liaison Group **(IRLG)** to implement their agreement. In January 1979, the Food Safety and Quality Service **(FSQS)**, Department of Agriculture, joined the IRLG.

These agencies recognized that although they often regulate the same chemicals, toxicity testing guidelines used by each agency are not always uniform. Among currently required tests, differences exist primarily in details of methodology and not in fundamental toxicological principles. The Testing Standards and Guidelines Work Group was established for the purpose of developing guidelines which would resolve existing differences and be used by all of the IRLG agencies for testing chemicals for health or environmental effects or both. This effort was to be coordinated with the development of other guidelines. On December 17, 1977, the Work Group held a public meeting to explain its purpose and goals, and to answer questions about its activities **(42 FR 59106, 15 November 1977)**.

IRLG guidelines are derived from those currently used or under development in the agencies, in international organizations, and in industry. Early drafts were circulated through each agency for review, and comments from that review are included in this guideline. In addition, drafts of the EPA Office of Pesticides Programs **(OPP)** proposed guidelines **(43 FR 37336, 22 August 1978)** under the Federal Insecticide, Fungicide and Rodenticide Act **(FIFRA)**, and public comments to those guidelines were used extensively in an effort to assure compatibility and to benefit from the appreciable time and effort the OPP staff put into their development. A second major source of information was *Principles and Procedures for Evaluating the Toxicity of Household Substances* **(NAS Pub. No. 1138)**, prepared for CPSC by the National Academy of Sciences. Still others heavily relied on were the Pharmaceutical Manufacturers' Association *Guidelines for the Assessment of Drug and Medical Device Safety in Animals* **(February 1977)**; the FDA, Bureau of Foods Direct Additive Cyclic Review Draft Guidelines; the *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*, Association of Food and Drug Officials of the U. S.; and protocols submitted by several industrial organizations in response to **43 FR 1987, 13 January 78**. The Work Group also used written and verbal comments received from the public in response to **44 FR 49015, 21 August 1979**. IRLG guidelines are being coordinated with development of guidelines by the Organization for Economic Cooperation and Development **(OECD)**. The Work Group acknowledges the contributions from each of these information sources and takes this opportunity to express its appreciation to the scientists who developed them and made them available.

Under most circumstances, the data obtained from this test should be sufficient to meet the requirements for a specific aspect of the toxicological characterization of the test substance. Under certain circumstances, however, the data may show a need for further study; or it may be recognized at the outset this guideline may have to be modified by an agency. It is important that tests be agreed upon between the investigator and the agency requiring them.

Each agency has the responsibility to decide what substance, and what form of the substance, it requires to be tested; which tests it will require; and how it will use the data derived from the tests.

## INTRODUCTION

This guideline is for use with substances given orally to the rat, mouse, hamster, or rabbit.

The purpose of this test is to yield data to help determine whether a test substance is potentially embryolethal and/or teratogenic. Treatment must be started early enough and continued long enough to include the period of organogenesis for the particular species used.

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## I. General Considerations

A. Good Laboratory Practices

Studies should be conducted according to good laboratory practice regulations (*e.g.*, "Nonclinical Laboratory Studies, Good Laboratory Practice Regulations," **43 FR 59986, 22 December 1978**).

B. Test Substance

1. The specific substance or mixtures of substances to be tested should be determined in consultation with the responsible agency. As far as is practical, composition of the test substance should be known. Information should include the name and quantities of all major components, known contaminants and impurities, and the percentage of unknown materials to account for 100% of the test samples.

2. Ideally, the lot of the substance tested should be the same throughout the study. The test sample should be stored under conditions that maintain its stability, strength, quality, and purity from the date of its production until the tests are complete.

C. Animals

1. Recommendations contained in DHEW pub. no. (NIH) 74-23, entitled *Guide for the Care and Use of Laboratory Animals*, should be followed for the care, maintenance, and housing of animals.

2. Healthy animals, not subjected to any previous experimental procedures, must be used.

3. The test animal shall be characterized as to species, strain, weight and/or age. Each animal must be assigned an appropriate identification number.

4. Animals should be individually caged for this test.

5. Animals must be assigned to groups in such a manner as to minimize bias and assure comparability of pertinent variables (for example, weight variation in animals used in a test should not exceed ± 20% of the mean weight).

6. Control animals must be housed, fed, and handled exactly like the test animals; and they must be caged to minimize contamination by the test substance.

D. Dead Animals, Necropsy, and Histopathology

Animals must be observed as necessary to insure that there is minimal loss due to cannibalism or management problems. Where possible, necropsy must be performed soon after an animal is sacrificed or found dead to minimize loss of tissues due to autolysis. When necropsy cannot be performed immediately, the animal must be refrigerated at temperatures low enough to minimize autolysis and not cause freezer burn. If histopathological examination is to be conducted, tissue specimens should be placed in appropriate fixative when they are taken from the animal.

## II. Specific Considerations

A. Test Preparation

1. Animals: Strain susceptibility to teratogens should be known. Strains with low fecundity should not be used. All test and control animals must be young, mature, pregnant females of uniform age, size, and parity.

2. Test groups: At least three test groups and one vehicle control group must be used. If there are insufficient data on the toxic properties of the vehicle used in administering the test substance, a sham control group should also be included. If no vehicle is used, then the controls should be sham treated.

3. Number of animals: Sufficient numbers of animals must be bred to assure that each test group and the vehicle control group will consist of at least 20 pregnant rats, mice, or hamsters, or at least 12 pregnant rabbits. The objective is to assure that sufficient pups are produced to permit evaluation of the teratogenic potential of the substance.

B. Test Procedure

1. Duration of test: Day 0 in the test is the day on which vaginal plug and/or sperm are observed (when feasible). The dose period should cover the period of major organogenesis. This may be taken as days 6-15 for rat and mouse, 6-14 for hamster, or 6-18 for rabbit. If day 0 is based on observation of mating or artificial insemination, the times stated should be adjusted by adding 1 day. Alternatively, the period of dosing may be extended to approximately 1 day before the expected delivery date.

   For substances that cause enzyme induction, or are highly toxic, shorter dosage periods may be appropriate.

   Fetuses shall be delivered by hysterotomy about one day prior to term.

2. Dosage: At least three dosage levels should be tested in addition to the controls. Unless limited by the physical/chemical nature, or biological effects of the test substance, the highest dosage level should induce evidence of maternal toxicity or affect fetal development. Maternal toxicity should not be so great as to compromise the integrity of the study (*e.g.*, 10% maternal deaths). Ideally, the intermediate dose(s) should induce some observable fetal effects attributable to the test substance. The low dosage level should not induce observable adverse effects attributable to the test substance. The dosage administered may be based on the individual animal's body weight on the first day of substance administration, or the animals may be weighed daily and the dosage adjusted accordingly.

3. Route of Administration: The test substance or vehicle should be administered by oral intubation at approximately the same time each day unless the physical/chemical characteristics, or pattern of human exposure to the test substance suggest a more appropriate route or time of administration.

4. Animal care: Food and water should be provided *ad libitum.* Pregnant females may be provided nesting materials, although it is not considered necessary.

5. Clinical observation: A careful clinical observation of each animal should be made at least once a day. Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study, *e.g.*, necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals. Any dam showing signs of imminent abortion or premature delivery should be sacrificed on the day such signs are observed. All toxicological and pharmacological signs should be recorded including time of onset, intensity, and duration. The time of death should also be noted. Individual records should be maintained for each animal.

6. Necropsy: Immediately after a dam is sacrificed, the uterus should be excised and examined for embryonic or fetal deaths and the number of live fetuses. The carcass of the dam should be weighed. When possible, the age of embryos or fetuses that die *in utero* should be estimated. The fetuses should be examined externally, weighed individually, and the weights recorded. The sex of each fetus should be determined if possible. In rodents about one half of each litter should be eviscerated, prepared, and examined for skeletal anomalies using the method of Staples (11) or equivalent. The remaining one half of each litter should be prepared and examined for soft tissue anomalies, using the method of Wilson (15) or equivalent. In rabbits, all fetuses should be examined by gross dissection for soft tissue anomalies and subsequently processed for skeletal examinations.

7. Statistical Analysis: Values from the control and test groups should be compared statistically. The following methods are suggested, but others may be substituted. Anomalies may be compared by chi-square methods or the binomial expansion method. Maternal body weight gains and weight of fetuses may be compared to those of controls by F-test and Student's t-test. Fetal survival and incidence of abnormalities per litter may be compared by nonparametric, rank-order methods.

## III. Data Reporting

A. Identification

Each test report should be signed by the persons responsible for the test and identify:

1. The laboratory where the test was performed by name and address;

2. The inclusive dates of the test; and

3. Each person primarily responsible for separate components of the test including (a) the conduct of the test, (b) pathology, (c) analysis of the data, (d) the writing of the report, and (e) any written or other matter contained in the report.

B. Body of Report

The test report must include all information necessary to provide a complete and accurate description and evaluation of the test procedures and results. Each report must include the following sections:

1. Summary and conclusions: This section of the test report should contain a brief description of the methods, a summary of the data, an analysis of the data, and a statement of the conclusions drawn. The summary must highlight all positive data or observations and any deviations from control data which may be indicative of toxic effects.

2. Materials: This section of the test report should include, but not be limited to, the following information:

   (a) Identification of the test substance, so far as practical, including:

      i. chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition, including names and quantities of known contaminants and impurities, listing the percentage of unidentifiable materials to account for 100% of the test sample;

      ii. manufacturer and lot number of the substance tested, and such information as physical state, pH, stability, and purity; and

      iii. specific identification of diluents, suspending agents, emulsifiers, or other materials used in administering the test substance.

   (b) Animal data, including:

      i. species and strain used and rationale for selection of the strain if other than a common laboratory strain;

      ii. source of supply of the animals;

      iii. description of any pre-test conditioning;

      iv. description of the method used in randomization of animals to test or control groups; and

v. numbers, age, and condition of dams in each test control group.

(c) Data on husbandry should include description of the caging conditions including number of animals per cage, diet, bedding material, ambient temperature, humidity, and lighting conditions.

3. Methods:

(a) Deviation from guidelines - This section shall indicate all ways in which the test procedure deviates from these guidelines and shall state the rationale for such deviation.

(b) Specification of test methods - This section shall include a full description of the experimental design and procedure, the length of the study, and the dates on which the study began and ended.

(c) Statistical analysis - All statistical methods used should be fully described or identified by reference.

(d) Data on dosage administration, including;

i. all dose levels administered, expressed as mg/kg of body weight;

ii. method, frequency, duration, and time of day; and

iii. total volume of substance (*i.e.,* test substance plus vehicle) contained in individual dosages.

(e) Data on observation methods, including:

i. duration; and

ii. method and frequency of observation of the animals.

4. Results:

The tabulation of data and individual results must accompany each report in sufficient detail to permit independent evaluation of results. The following information should be included:

(a) Time of observation of each abnormal sign and its subsequent course.

(b) Age (or weight) at the start of the test.

(c) Body weights and body weight change based on the carcass weight.

(d) Signs of resorptions.

(e) Toxic response data.

(f) Time of death.

(g) Pregnancy and litter data.

(h) Fetal data (live/dead, soft tissue, skeletal defects and identification of fetal dam).

Evaluation of the results should include:

(a) an evaluation of the relationship, if any, between exposure to the test substance and the anomalies, and

(b) an indication of the dosage level at which no toxic effects attributable to the test substance appeared.

## IV. Suggested Reading


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The IRLG Testing Standards and Guidelines Work Group dedicates its guidelines to the memory of Dr. William D'Aguanno, whose commitment and talent contributed immeasurably to their development.